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Microplastics in Sewage Sludge: Effects of Treatment

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Abstract

Waste Water Treatment Plants (WWTPs) are receptors for the cumulative loading of microplastics (MPs) derived from industry, landfill, domestic waste water and storm water. The partitioning of MPs through the settlement processes of waste water treatment results in the majority becoming entrained in the sewage sludge. This study characterised MPs in sludge samples from seven WWTPs in Ireland, which use anaerobic digestion (AD), thermal drying (TD), or lime stabilisation (LS) treatment processes. Abundances ranged from 4,196 to 15,385 particles kg^{-1} (dry weight). Results of a general linear mixed model (GLMM) showed significantly higher abundances of MPs in smaller size classes in the LS samples, suggesting that the treatment process of LS shear MP particles. In contrast, lower abundances of MPs found in the AD samples suggest that this process may reduce MP abundances. Surface morphologies examined using Scanning Electron Microscopy (SEM) showed characteristics of melting and blistering of TD MPs and shredding and flaking of LS MPs. This study highlights the potential for sewage sludge treatment processes to affect the risk of

MP pollution prior to land spreading and may have implications for legislation governing the application of biosolids to agricultural land.

Keywords: Microplastics; sewage sludge; biosolids; anaerobic digestion; lime stabilisation; thermal drying.

1. Introduction

Microplastics (MPs) are synthetic polymers measuring less than 5 mm in diameter and are derived from a wide range of sources including synthetic fibres from clothing,[1,2] polymer manufacturing and processing industries,[3] and personal care products. [4] They have the potential to adsorb persistent organic contaminants[5,6] and priority metals[7-11] from the surrounding environment. These may be released upon digestion by biota or through environmental degradation, leading to possible impacts to human health and ecosystems.[12-14] Over the last 10 years, many studies have investigated the distribution[1,15] and effects[16-19] of MPs within the marine environment. Indeed, MPs have been found in Polar Regions[20] and in a range of freshwater environments worldwide.[21-24] Despite this, few studies have sought to determine land-based sources of MPs.[25] Wastewater treatment plants (WWTPs) have been identified as receptors of MP pollution and effective in capturing the majority of MPs in the sludge during settlement regimes[26], as first found by Habib et al. (1998) when they used synthetic fibers as a proxies for the presence of sewage.[27] More than 10 million tonnes of sewage sludge was produced in WWTPs in the European Union (EU) in 2010.[27] European Union policy on sustainability and recycling of resources[28] favours the recycling of sludge. The introduction of EU legislation such as the Landfill Directive[29] and the Renewable Energy Directive[30] have diverted sewage sludge from landfill and incineration into use for energy production[31] and agriculture.[32] In some countries, such as Ireland, up to 80% of municipal wastewater sludge is reused in agriculture.[33,34] Guidelines stipulate that the sludge must undergo some type of treatment (after which it is commonly referred to as 'biosolids') prior to land application. This may include lime stabilisation (LS), anaerobic digestion (AD), composting, or thermal drying (TD).[31] As approximately 99% of MPs are retained in sewage sludge generated in WWTPs, [35] there is a possibility that land applied sludge, even having undergone treatment, could be a source of MP pollution.

The regulations for the use of biosolids in the EU and USA stipulate limit levels for pathogen content, maximum metal and nutrient application rates to land,[36] and vector (flies and rodents) attraction reduction (USA only). Restrictions in land application of biosolids vary between the EU and USA. Under US federal legislation, the application of biosolids to agricultural land can occur without restriction in volume or duration, if the contamination level reaches an exceptional quality "EQ".[37] In Europe, sewage sludge is dealt with very differently among member states, and application to land is banned in some countries.[38-40]

As most sewage sludge undergoes treatment prior to land-spreading, the effects of these treatments on MP morphology is important but remains largely unknown, with some evidence of increased abundance of fibres at a smaller size range for LS sludge[41] which is probably due to alkaline hydrolysis.[42] Therefore, the aim of this study was to investigate the first stage of the MP pathway post-WWTP, and the impacts of different treatments. In particular, it aimed to determine if (1) MPs are present in treated sewage sludge from a range of WWTPs employing AD, TD and LS as treatment techniques, and (2) the type of treatment used (TD, AD, LS) employed at the WWTP impacts on MP abundance and characteristics, including size and surface morphology.

2. Methodology

2.1 WWTP sludge sample collection and preparation

Sewage sludge, having undergone treatment including TD, AD or LS, was collected from seven waste WWTPs with population equivalents (PEs) ranging from 6500 to 2.4 million (Table 1). These WWTPs received waste water from industry, storm water run-off and domestic sources, all of which comprised up to 30% of the influent organic loading (measured as biochemical oxygen demand, BOD) (Table1). Three replicate samples of 30 g were obtained from each WWTP and stored at -20^{o}C prior to sample preparation. The treated sewage sludge had dry matter (DM) contents ranging from 24% (AD) to 87% (TD). Pellets of TD sludge were placed in water for 1 week to induce softening, transferred to a water bath (30^{o}C) for 24 hr, and placed in an "end-over-end" shaker (Parvalux, UK) for 12 hr. This shaking procedure was repeated until the pellets were sufficiently softened without compromising the physical characteristics of the MPs. The samples were subsequently washed through a 250 µm sieve, which resulted in complete degradation of the pelleted clumps prior to elutriation. A proportion of the washed through fraction was retained and passed through 212, 63, and 45 µm sieves for particle size determination or particle size fractionation.

Anaerobically digested and LS sludge were soaked in filtered tap water to soften and homogenise them, and were also washed through 250, 212, 63 and 45 µm sieves to determine particle size fractions. As the LS sludge had an oily appearance, thought to be derived from the break-down of cellulosic material through alkaline hydrolysis, it was decided that the elutriation and other density separation techniques were unsuitable for extraction of MPs.

Instead, 10 g from each replicate sample were examined by passing it directly through a filter (GF/C: Whatman ™, 1.2 µm) using vacuum filtration.

2.2. Microplastics Extraction

2.2.1 Elutriation

The principal of elutriation was used as the first step in the separation of MPs from other sample components. Elutriation separates lighter particles from heavier ones through an upward flow of liquid and/or gas, and has been widely used in the separation of biota within sediment samples.[42] To separate MPs from the sludge samples, an elutriation column, based on the design of Claessens et al.[43] was constructed.

2.2.1.1 Column extraction efficiency estimation

To check for efficiency of the column in extracting MP, three sediment samples, each weighing 40 g, were spiked with 50 MP particles of high density polyethylene (HDPE) (three colours) and PVC, and run through the column. The HDPE samples used were shavings of approximately 1.0 (L) × 4.0 (W) × 2.0 mm (B). The PVC particles were of a similar dimension, but were more brittle. Therefore, each particle was marked with a blue marker to ensure that particles were not counted twice upon recovery. The number of particles, separated from the sediment matrix, that exited the column, was enumerated and the percentage efficiency was calculated.

2.2.2. Zinc chloride ($ZnCl_2$) extraction

The MP extraction was filtered through 250 µm mesh, rinsed into a separatory funnel with 1 molar $ZnCl_2$ solution, and brought to a volume of 300 ml. The funnel was plugged, vigorously shaken for 1 min, and allowed to settle (20 min). The settled material was drained and the remainder of the sample was filtered onto glass fibre filters (GF/C: Whatman ™, 1.2 µm). The oily appearance of the LS samples rendered this density separation technique unsuitable for extraction of MP.

2.3. Characterisation of MPs

The filters were examined using stereomicroscopy equipped with a polariser (Olympus SZX10) attachment and a Qimaging® Retiga™ 2000R digital camera. Microplastics were identified and enumerated based on several criteria including form, colour and sheen used in previous studies as described by Hidalgo Ruz et al. [44] The form of a synthetic fibre should not taper at either end, while not having a rigidly straight form. Any polymer will not have cellular structure or other organic structures. Artificial fibre particles also have uniformity of colour and exhibit a sheen once passed through the polarized light. Where ambiguity remained following these observations, the suspected polymer was manipulated with a hot pin by which a melted form indicated a positive result. Microplastics were measured and allotted to the following size categories: 250-400 µm, 400-600 µm, 600-1000 µm, and 1000-4000 µm. Suspected MPs were enumerated and measured, and approximately 10% of MP samples from each filter paper were set aside for polymer identification. Microplastics for which any ambiguity remained as to if it was a polymer, were automatically selected for analyses.

Attenuated total reflectance (ATR) and Fourier transform infrared spectroscopy (FTIR) (Perkin Elmer, USA, Spectrum Two™ with Universal ATR Accessory and Thermo Scientific, UK, Nicolet iN10 FTIR microscope with germanium Tip Slide-on-ATR) were used to analyse approximately 10% of MP samples. The spectra were obtained with 3-second data collection (16 scans per sample) over the wave number range 600 – 4000 cm^{-1} using a liquid nitrogen-cooled MCT-A detector at 8 cm^{-1} resolution. Microplastic samples extracted from the sludge (and pristine plastics for comparative purposes) were gold-coated (Emitecg K550, Quorum technologies, Ltd., UK) and subjected to variable pressure scanning electron microscopy (SEM) in secondary electron mode using a Hitachi model S2600N (Hitachinaka, Japan). The analyses were performed at accelerating voltages of 10 - 20 kv, an emission current (I_c) of 10 μA, and a working distance of 12 - 24mm.[44]

2.4 Quality control and contamination prevention

Cotton laboratory coats and nitrile gloves were used during the sample preparation and analyses. In addition, synthetic clothing was avoided and samples were covered at all times and working surfaces were cleaned with alcohol prior to use. When analysing filter papers, a blank filter paper was exposed to the open laboratory conditions to assess the possibility of air-borne contamination.

2.5. Data analyses

Statistical analyses were carried out using Minitab 17 (2010) and R.[45] As data were not normally distributed, non-parametric tests were used to test for differences in MP abundances amongst locations (Mann-Whitney Test). To investigate if there were any possible effects of PE on abundance, a Spearman's rank correlation analysis test was utilised. With the exception of one WWTP, there was only one treatment method employed per site (Table 1), so in-site correlation was not possible. Each site was treated as an independent measurement and plotted using a box plot. A generalised linear mixed effect model (GLMM) was used (Eqn. 1) to investigate the high number of MP particles in the smaller class sizes at WWTPs in which LS was employed.

$$\text{Microplastic counts} = \text{Treatment Type} + \text{Population Equivalent} + \frac{1}{\text{Treatment Plant}}$$

Eqn. 1

Where *1/Treatment Plant* specifies a random intercept model.

A separate GLMM for each size class was carried out using a Poisson distribution and a random effect term to account for nesting of replicates within WWTPs to determine which explanatory variable was responsible for larger proportions of smaller MP particles at WWTPs in which LS was employed.

3. Results and Discussion

3.1 Characterisation of treated sewage sludge

The characteristics of the sewage sludge treated using AD, LS and TD had varying physical characteristics. The particle size fractionation (g/kg) of the AD samples was smaller than the LS and TD samples (Table 2), and had a sandy appearance. The AD samples were very dark and heavy with some cellulosic material, whereas the TD samples had a lot of cellulosic material entrained, which was difficult to separate during elutriation and zinc chloride extraction. Although this cellulosic material was distinctive from MP material (in that its fibres tapered at the ends and it was often branched) and therefore easy to disqualify, its presence in the samples greatly increased the time and consumables (filter papers) utilised during the filtration process. High levels of cellulose derived from toilet paper in sewage may merit the inclusion of a digestion process using the cellulase enzyme, as has been previously used for the isolation of MPs in North Sea sediments.[46]

3.2 Microplastics Extraction

3.2.1 Elutriation column extraction efficiency estimation

The average extraction efficiency rate of the elutriation column for the spiked sediment samples was 90%, 94% and 91% for the red, blue and black HDPE particles, respectively. The elutriation process was less efficient for the PVC particles, which resulted in an average extraction efficiency of 80%. This is an indication that results of MP abundance in this study may be an underestimation. As the efficiency test was conducted only for fragments at one size only, it may not be representative of efficiency of fibre removal.

3.3 Characterisation of Microplastics

3.3. 1 Microplastics abundance

Microplastics extracted from the biosolids ranged from an average of 4,196 to 15,385 particles kg^{-1} (DM) among the seven sites, with significant differences in MP abundances between some sites and within Site 1 (1A, 1B) between AD samples and TD samples (Mann Whitney, $w = 15$, $p = 0.0809$; Figure 1). This is likely to be an underestimation due to losses in column efficiency (approx. 20%) and through the use of a 250 µm sieve from which a proportion of fibres may be lost. The abundances found in this study are in the same order of magnitude to the study by Zubris et al.[42] who reported between 3,000 and 4,000 particles kg^{-1}. In the current study, a lack of correlation between PE and MP abundance kg^{-1} (Spearman's rank, $r = -0.308$, $p = 0.458$) implies that these differences may have been due to the variation of input sources (industrial, storm water, landfill etc.). However, as no data exist for the temporal variation of MPs in sewage sludge, there is a possibility that these variations are a result of fluxes in MP input, which could be a result of peak MP emission times in relation to household or industrial activity. The significantly lower abundance of MPs in an anaerobically digested biosolid sample compared to all other sample except Site 3, which was also treated with AD, posits an interesting question over the possible role of AD in the degradation of polymers collected from the same site as sample 1A (taken roughly at the same time). Without pre-treatment samples, there is no evidence to prove that the mesophilic anaerobic digestion (MAD) used at the AD WWTPs in this study, facilitated the breakdown of MPs. Indeed, few studies have examined the breakdown of polymers in anaerobic digesters. However, one pilot study investigated the effect of plastic waste on the functioning of anaerobic digestion and found that digesters from which plastic was removed, produced less gas than those to which plastic was added.[47] As there is already substantial evidence of microbial breakdown of polymers through the activity of exoenzymes (promoting depolymerisation) and assimilation of smaller articles resulting in mineralisation, [49 50,51] the role of degradation by microorganisms within the AD systems should be further investigated.

3.3.2 Morphological categorization and polymer identification of microplastics

This study confirmed that MPs are retained in the sewage sludge and are largely composed of fibres, similar to what was found by Talvite et al.[46] and Magnusson and Norén.[35] Approximately 75.8% of the MP consisted of fibres, followed by fragments, films, other unidentified particles, and spheres, which accounted for only 0.3% of total MP abundance (Table 3). The greatest proportion of MP fragments was found at the LS WWTPs, with Site 6 being the only site to have marginally more fragments than fibres (Table 3; Figure 2). Polymers, identified by FTIR, comprised HDPE, polyethylene (PE) polyester, acrylic, polyethylene terephthalate (PET), polypropylene, and polyamide (Figure 3). Some of these contained minerals. Although waste water derived from households generate high quantities of fibres, principally derived from clothes washing of >1900 fibres per wash[1], other industrial sources of fibres such as the fibre manufacturing industry may also be important contributors.

3.3.3. Size of micrplastics

Using the fitted coefficients from the GLMM, a study hypotheses of no difference between all pairwise combinations of the treatment effects were tested. At small and medium particle sizes, the LS treatment was significantly different from both TD and AD treatments (Figure 4; $P < 0.001$; sizes classes A and C; $P < 0.05$ size class B). The larger number of smaller MP particles in LS samples corresponded with the larger proportion of smaller particle sizes determined from the particle size fractionation. As it was not possible to obtain pre-treatment samples, it is not possible to wholly assign the differences in size classes to the treatment processes. However, the elevated numbers at the small size classes for LS samples are in agreement with results reported by Zubris and Richards [42] , where there was some evidence of elevated abundance of MPs at smaller size classes. Further investigations are required to investigate accelerated proliferation of MP pollution through sludge treatment processes.

3.3.4 Surface morphologies of microplastics

Scanning electron micrographs of surface textures of polymers entrained in the treated biosolids had some surface morphologies, which varied among treatment type. An unknown polymer fibre, which was thermally dried, had distinct blistering and fracturing, particularly in the fibre curves (Figure 5: A-C). Additionally, polymer fragments from TD samples, identified as HDPE and PE fragments, showed wrinkling, melding and some fracturing, which was quite distinct from pre-treatment samples (Figure 6: G-I; Figure 7: D-F). Surface morphologies of MPs originating from LS biosolids had a more shredded and flaked appearance for the unknown polymer (Figure 5: D-F) and a HDPE sample (Figure 5: D-F). Anaerobically digested samples of an unknown polymer had deep cleavage, which was distinct from any other observations (Figure 5: G-I).

4. Conclusions

Although it was not possible to assign wholly the abundances or size distributions to the treatment processes, results suggest that treatment processes may have an effect. If MPs are altered by treatment, the potential for impact may also be influenced depending accordingly. This could add to the unknown risks associated with MPs in sewage sludge. Regardless of treatment regimes, over time, there may be consequences for the accumulation of MPs in terrestrial, freshwater, or marine ecosystems derived from land-spreading of sewage sludge or biosolids.

Microplastics entrained in biosolids which are applied to land, may be degraded through photo-degradation and thermo-oxidative degradation[49,53] exacerbating the problem of land-spread MP pollution. The interaction of MPs with contaminants in the soil, could have major consequences for the absorption and transportation of contamination elsewhere. Surface weathering and the subsequent attachment of organic matter and the resulting negative charge attracts metals including cadmium, lead and zinc.[53] Whether agricultural land is a sink or a source of MP pollution remains unclear. Microplastic fibres have been found on land 15 years post application, and some evidence of vertical translocation through the soil has also been found.[41] Possible impacts arising from land-applied MPs begin in the terrestrial ecosystem with implications for terrestrial species such as earth worms[55] and birds feeding on terrestrial ecosystems.[56] As legislation in the EU and the US generally permit the land application of sewage sludge, there is a strong possibility that large amounts of MPs are emitted to freshwater, where currently little is known about their impacts on species and habitats.[57] Furthermore, buffer zones around freshwater bodies, which may be stipulated in "codes of good practice", do not take into account the mechanisms of transportation of MP vertically through the soil or with surface runoff following a precipitation event. While legislation currently takes into account pathogens as well as nutrient and metal concentrations of treated sludge,[58] it does not consider the presence of MPs within the sludge, and their associated risks. The predicted exponential growth of the plastics industry for the coming years[59] may be accompanied by a significant increase in MPs in the waste stream. Therefore, vigilant management of cumulative sources of MPs such as sewage sludge or biosolids is necessary. In particular, this study has highlighted the potential for treatment processes to alter the counts of MPs which, in turn, increases the available area for absorption/desorption of organic pollutants.

A review of sewage sludge treatment processes and their implications for MP pollution should be more thoroughly investigated, with before and after treatment comparisons. In particular, the role of degradation by microorganisms within the AD systems should be further investigated. Knowledge gaps regarding the factors critical for the mobilisation and transport of MPs likely to affect the pathway of land-spread sewage sludge MP pollution should to be addressed in order to determine MP flow within the terrestrial system and to freshwater systems. Only when experimental data are acquired, can we estimate exposure and associated risks to the environment from MP pollution.

Supporting Information

Detailed description of the dimensions of the elutriation column, accompanied by a photograph and schematic representation. Flow rates and technique used for extraction of MPs using the elutriation column are also included.

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Bibliography

(1) Browne, M. A.; Crump, P.; Niven, S. J.; Teuten, E.; Tonkin, A.; Galloway, T.; Thompson, R. Accumulation of Microplastic on Shorelines Woldwide: Sources and Sinks. *Environ. Sci. Technol.* **2011**, *45* (21), 9175–9179.

(2) Astrom, L. Shedding of synthetic microfibers from textiles. University of Gothenburg, Sweden 2016.

(3) Lechner, A.; Ramler, D. The discharge of certain amounts of industrial microplastic from a production plant into the River Danube is permitted by the Austrian legislation. *Environ. Pollut.* **2015**, *200C*, 159–160.

(4) Fendall, L. S.; Sewell, M. A. Contributing to marine pollution by washing your face: microplastics in facial cleansers. *Mar. Pollut. Bull.* **2009**, *58* (8), 1225–1228.

(5) Teuten, E. L.; Saquing, J. M.; Knappe, D. R. U.; Barlaz, M. A.; Jonsson, S.; Björn, A.; Rowland, S. J.; Thompson, R. C.; Galloway, T. S.; Yamashita, R.; et al. Transport and release of chemicals from plastics to the environment and to wildlife. *Philos. Trans. R. Soc. London B Biol. Sci.* **2009**, *364* (1526), 2027–2045.

(6) Engler, R. E. The Complex Interaction between Marine Debris and Toxic Chemicals in the Ocean. *Environ. Sci. Technol.* **2012**, *46* (22), 12302–12315.

(7) Ashton, K.; Holmes, L.; Turner, A. Association of metals with plastic production pellets in the marine environment. *Mar. Pollut. Bull.* **2010**, *60* (11), 2050–2055.

(8) Holmes, L. A.; Turner, A.; Thompson, R. C. Adsorption of trace metals to plastic resin pellets in the marine environment. *Environ. Pollut.* **2012**, *160*, 42–48.

(9) Nakashima, E.; Isobe, A.; Kako, S.; Itai, T.; Takahashi, S. Quantification of Toxic Metals Derived from Macroplastic Litter on Ookushi Beach, Japan. *Environ. Sci. Technol.* **2012**, *46* (18), 10099–10105.

(10) Rochman, C. M.; Hentschel, B. T.; Teh, S. J. Long-Term Sorption of Metals Is Similar among Plastic Types: Implications for Plastic Debris in Aquatic Environments. *PLoS One* **2014**, *9* (1), e85433.

(11) Brennecke, D.; Duarte, B.; Paiva, F.; Caçador, I.; Canning-Clode, J. Microplastics as vector for heavy metal contamination from the marine environment. *Estuar. Coast. Shelf Sci.* **2016**, *178*, 189–195.

(12) Cooper, D. A.; Corcoran, P. L. Effects of mechanical and chemical processes on the degradation of plastic beach debris on the island of Kauai, Hawaii. *Mar. Pollut. Bull.* **2010**, *60* (5), 650–654

(13) Andrady, A. L. Microplastics in the marine environment. *Mar. Pollut. Bull.* **2011**, *62* (8), 1596–1605.

(14) Bouwmeester, H.; Hollman, P. C. H.; Peters, R. J. B. Potential Health Impact of Environmentally Released Micro- and Nanoplastics in the Human Food Production Chain: Experiences from Nanotoxicology. *Environ. Sci. Technol.* **2015**, *49* (15), 8932–8947.
(15) Cole, M.; Lindeque, P.; Halsband, C.; Galloway, T. S. Microplastics as contaminants in the marine environment: a review. *Mar. Pollut. Bull.* **2011**, *62* (12), 2588–2597.
(16) von Moos, N.; Burkhardt-Holm, P.; Köhler, A. Uptake and Effects of Microplastics on Cells and Tissue of the Blue Mussel Mytilus edulis L. after an Experimental Exposure. *Environ. Sci. Technol.* **2012**, *46* (20), 11327–11335.
(17) Cole, M.; Lindeque, P.; Fileman, E.; Halsband, C.; Goodhead, R.; Moger, J.; Galloway, T. S. Microplastic Ingestion by Zooplankton. *Environ. Sci. Technol.* **2013**, *47* (12), 6646–6655.
(18) Remy, F.; Collard, F.; Gilbert, B.; Compère, P.; Eppe, G.; Lepoint, G. When Microplastic Is Not Plastic: The Ingestion of Artificial Cellulose Fibers by Macrofauna Living in Seagrass Macrophytodetritus. *Environ. Sci. Technol.* **2015**, *49* (18), 11158–11166.
(19) Watts, A. J. R.; Urbina, M. A.; Goodhead, R. M.; Moger, J.; Lewis, C.; Galloway, T. S. Effect of microplastic on the gills of the Shore Crab Carcinus maenas. *Environ. Sci. Technol.* **2016**.
(20) Lusher, A. L.; Burke, A.; O'Connor, I.; Officer, R. Microplastic pollution in the Northeast Atlantic Ocean: Validated and opportunistic sampling. *Mar. Pollut. Bull.* **2014**, *88* (1), 325–333.
(21) Eriksen, M.; Mason, S.; Wilson, S.; Box, C.; Zellers, A.; Edwards, W.; Farley, H.; Amato, S. Microplastic pollution in the surface waters of the Laurentian Great Lakes. *Mar. Pollut. Bull.* **2013**, *77* (1–2), 177–182.
(22) McCormick, A.; Hoellein, T. J.; Mason, S. A.; Schluep, J.; Kelly, J. J. Microplastic is an Abundant and Distinct Microbial Habitat in an Urban River. *Environ. Sci. Technol.* **2014**, *48* (20), 11863–11871.
(23) Castañeda, R. A.; Avlijas, S.; Simard, M. A.; Ricciardi, A. Microplastic pollution in St. Lawrence River sediments. *Can. J. Fish. Aquat. Sci.* **2014**, *71* (12), 1767–1771.
(24) Free, C. M.; Jensen, O. P.; Mason, S. A.; Eriksen, M.; Williamson, N. J.; Boldgiv, B. High-levels of microplastic pollution in a large, remote, mountain lake. *Mar. Pollut. Bull.* **2014**, *85* (1), 156–163.
(25) Lechner, A.; Ramler, D. The discharge of certain amounts of industrial microplastic from a production plant into the River Danube is permitted by the Austrian legislation. *Environ. Pollut.* **2015**, *200C*, 159–160.
(26) Carr, S. A.; Liu, J.; Tesoro, A. G. Transport and Fate of Microplastic Particles in Wastewater Treatment Plants. *Water Res.* **2016**, *91*, 174–182.
(27) Habib, D.; Locke, D. C.; Cannone, L. J. Synthetic Fibers as Indicators of Municipal Sewage Sludge, Sludge Products, and Sewage Treatment Plant Effluents. *Water. Air. Soil Pollut.* **1998**, *103* (1), 1–8.
(28) Eurostat (2014) Sewage sludge production and disposal. http://appsso.eurostat.ec.europa.eu/nui/show.do?dataset=env_ww_spd&lang=en
(29) Commission of the European Communities (COM) (2014) Towards a circular economy: a zero waste programme for Europe. http://eur-lex.europa.eu/resource.html?uri=cellar:aa88c66d-4553-11e4-a0cb-01aa75ed71a1.0022.03/DOC_1&format=PDF
(30) EC, 1999. Economic Commission. Council Directive of the 26 April 1999 on landfill waste. (1999/31/EC) Available at: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A31999L0031
(31) EC, 2009. Directive 2009/28/EC of the European parliament and of the Council of 23 April 2009 on the promortion of use of energy from renewable sources and amending subsequently repealing Directives 2001/77/EC and 2003/30/EC.

(32) Gikas, P. Electrical energy production from biosolids: a comparative study between anaerobic digestion and ultra-high-temperature gasification. Environ. Technol. **2014** *35*, 2140-2146.

(33) Healy, M.G., Clarke, R., Peyton, D., Cummins, E., Moynihan, E.L., Martins, A., Beraud, P., Fenton, O. Resource recovery from sludge. p. 139 – 162. In: K. Konstantinos and K.P. Tsagarakis, Eds.) Sewage treatment plants: economic evaluation of innovative technologies for energy efficiency. IWA, London, 2015. ISBN: 9781780405018.

(34) EPA. Urban waste water treatment in 2014. http://www.epa.ie/pubs/reports/water/wastewater/2014%20waste%20water%20report_web.pdf

(35) Eurostat 2016. Sewage Sludge production and disposal. http://appsso.eurostat.ec.europa.eu/nui/submitViewTableAction.do.

(36) Magnusson K., Norén F., 2014. Screening of microplastic particles in and downstream from a wastewater treatment plant. Report to the Swedish Environmental Research Institute: C55.

(37) Lucid, J. D., Fenton, O., Grant, J., & Healy, M. G. Effect of Rainfall Time Interval on Runoff Losses of Biosolids and Meat and Bone Meal when Applied to a Grassland Soil. *Water Air Soil Pollut*. **2014**, *225* (8), 1-11.

(38) Harrison, E. Z.; McBride, M. B.; Bouldin, D. R. Land application of sewage sludges: an appraisal of the US regulations. *Int. J. Environ. Pollut.* **1999**, *11* (1), 1–36.

(39) Environmental, economic and social impacts of the use of sewage sludge on land. Final report- Part I; Overview Report. European Commission Service Service Contract. No.070307/2008/517358/ETU/G4; 2013.

(40) Environmental, economic and social impacts of the use of sewage sludge on land. Final report- Part I; Report on Options and Impacts. European Commission Service Contract No.070307/2008/517358/ETU/G4; 2013.

(41) Environmental, economic and social impacts of the use of sewage sludge on land. Final report- Part I; Project Interim Reports. European Commission Service Contract No.070307/2008/517358/ETU/G4; 2013.

(42) Zubris, K. A. V; Richards, B. K. Synthetic fibers as an indicator of land application of sludge. *Environ. Pollut.* **2005**, *138* (2), 201–211.

(43) Walling, D. E.; Woodward, J. C. Use of a field-based water elutriation system for monitoring the in situ particle size characteristics of fluvial suspended sediment. *Water Res.* **1993**, *27* (9), 1413–1421.

(44) Claessens, M.; Van Cauwenberghe, L.; Vandegehuchte, M. B.; Janssen, C. R. New techniques for the detection of microplastics in sediments and field collected organisms. *Mar. Pollut. Bull.* **2013**, *70* (1-2), 227–233.

(45) Morrison, L., Feely, M., Stengel, D. B., Blamey, N., Dockery, P., Sherlock, A. and Timmins, É. Seaweed attachment to bedrock: biophysical evidence for a new geophycology paradigm. *Geobiol*. **2009** 7: 477-487 (DOI: 10.1111/j.1472-4669.2009. 00206.x).

(46) Lorenz, C. (2014): Detection of microplastics in marine sediments of the German Coast via FT-IR spectroscopy, Master thesis, Universität Rostock.

(47) Morrison, L.; Feely, M.; Stengel, D. B.; Blamey, N.; Dockery, P.; Sherlock, A.; Timmins, E. Seaweed attachment to bedrock: biophysical evidence for a new geophycology paradigm. *Geobiology* **2009**, *7* (4), 477–487.

(48) Talvitie, J.; Heinonen, M.; Pääkkönen, J.-P.; Vahtera, E.; Mikola, A.; Setälä, O.; Vahala, R. Do wastewater treatment plants act as a potential point source of microplastics?

Preliminary study in the coastal Gulf of Finland, Baltic Sea. *Water Sci. Technol.* **2015**, *72* (9), 1495–1504.

(49) Kamal, M.R, Huang, B. Handbook of Polymer Degradation, Marcek Dekker, New York, 127-168, 1992.

(50) Shah, A. A.; Hasan, F.; Hameed, A.; Ahmed, S. Biological degradation of plastics: a comprehensive review. *Biotechnol. Adv.* **2008**, *26* (3), 246–265.

(51) Andrady, A. L.; Hamid, S. H.; Hu, X.; Torikai, A. Effects of increased solar ultraviolet radiation on materials. *J. Photochem. Photobiol. B Biol.* **1998**, *46* (1-3), 96–103.Gu, J.-D. Microbiological deterioration and degradation of synthetic polymeric materials: recent research advances. *Int. Biodeterior. Biodegradation* **2003**, *52* (2), 69–91.

(52) Yoshida, S.; Hiraga, K.; Takehana, T.; Taniguchi, I.; Yamaji, H.; Maeda, Y.; Toyohara, K.; Miyamoto, K.; Kimura, Y.; Oda, K. A bacterium that degrades and assimilates poly(ethylene terephthalate). *Science* **2016**, *351* (6278), 1196–1199.

(53) Artham, T.; Doble, M. Biodegradation of Aliphatic and Aromatic Polycarbonates. *Macromol. Biosci.* **2008**, *8* (1), 14–24.

(54) Contat-Rodrigo, L. Thermal characterization of the oxo-degradation of polypropylene containing a pro-oxidant/pro-degradant additive. *Polym. Degrad. Stab.* **2013**, *98* (11), 2117–2124.

(55) Turner, A.; Holmes, L. A. Adsorption of trace metals by microplastic pellets in fresh water. *Environmental Chemistry*. 2015, pp 600–610.

(56) Peyton, D.P., Healy, M.G., Fleming, G.T.A., Grant, J., Wall, D., Morrison, L., Cormican, M., Fenton, O. Nutrient, metal and microbial loss in surface runoff following treated sludge and dairy cattle slurry application to an Irish grassland soil. *Sci Total Environ*. **2016**. *541*, 218-229.

(57) Huerta Lwanga, E.; Gertsen, H.; Gooren, H.; Peters, P.; Salánki, T.; van der Ploeg, M.; Besseling, E.; Koelmans, A. A.; Geissen, V. Microplastics in the Terrestrial Ecosystem: Implications for Lumbricus terrestris (Oligochaeta, Lumbricidae). *Environ. Sci. Technol.* **2016**, *50* (5), 2685–2691.

(58) Zhao, S.; Zhu, L.; Li, D. Microscopic anthropogenic litter in terrestrial birds from Shanghai, China: Not only plastics but also natural fibers. *Sci. Total Environ.* **2016**, *550*, 1110–1115.

(59) Eerkes-Medrano, D.; Thompson, R. C.; Aldridge, D. C. Microplastics in freshwater systems: a review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. *Water Res.* **2015**, *75*, 63–82.

(60) Healy, M. G.; Fenton, O.; Forrestal, P. J.; Danaher, M.; Brennan, R. B.; Morrison, L. Metal concentrations in lime stabilised, thermally dried and anaerobically digested sewage sludges. *Waste Manag.* **2016**, *48*, 404–408.

(61) Plastics Europe. An Analysis of European plastic production, demand and waste data for 2011. Brussels, Belguim, 2012.

Table 1. Characteristics of municipal wastewater treatment sites investigated (adapted from Healy et al., 2016)

Site	WWTP/ agglomeration size (PEs)	Landfill leachate as % of influent BOD load	Industrial, and domestic septic tank sludge[1] as % of influent BOD load	Type of treatment
1A	2,362,329	<0.01	<0.01	Thermal drying, anaerobic digestion
1B	284,696	0.3	24	Thermal drying
2	179,000	unknown	30	Anaerobic digestion
3	130,000	unknown	0.008	Thermal drying
4	101,000	2.0	unknown	Lime stabilisation
5	31,788	0.25	unknown	Lime stabilisation
6	25,000	0.7	0	Thermal drying
7	6,500	Unknown	Unknown	Thermal drying

[1] Most recent available figures in all WWTPs (2013)

Table 2. Particle size fraction (g) of lime stabilised (LS), anaerobically digested (AD) and thermally dried (TD) samples (40 g).

Size fraction	Treatment type		
	LS	AD	TD
> 212 µm	3.004 ± 0.550	31.753± 0.578	35.503± 0.661
> 63 µm	27.410± 0.840	7.948± 0.7778	3.593± 0.894
> 45 µm	9.400± 1.166	0.327± 0.241	0.930± 0.486
< 45 µm	0.200 ± 0.213	0.000± 0.00	0.000± 0.000

Table 3. Breakdown of types of average microplastic abundance kg^{-1} (dry matter) among sites.

		Microplastic Types				
Site no.	Treatment	Fibres	Fragments	Films	Spheres	other
1A	TD	9,113	511	255	89	44
1B	AD	2,065	611	67	0	0
2	TD	5,583	588	222	44	67
3	AD	4,007	855	111	33	150
4	TD	13,675	1,143	366	33	178
5	LS	10,778	3,075	122	11	78
6	LS	4,762	5,228	11	0	11
7	TD	3,463	511	167	0	56
Total	**-**	**53,447**	**12,521**	**1,321**	**211**	**583**
%	-	78.5	18.4	1.9	0.3	0.9

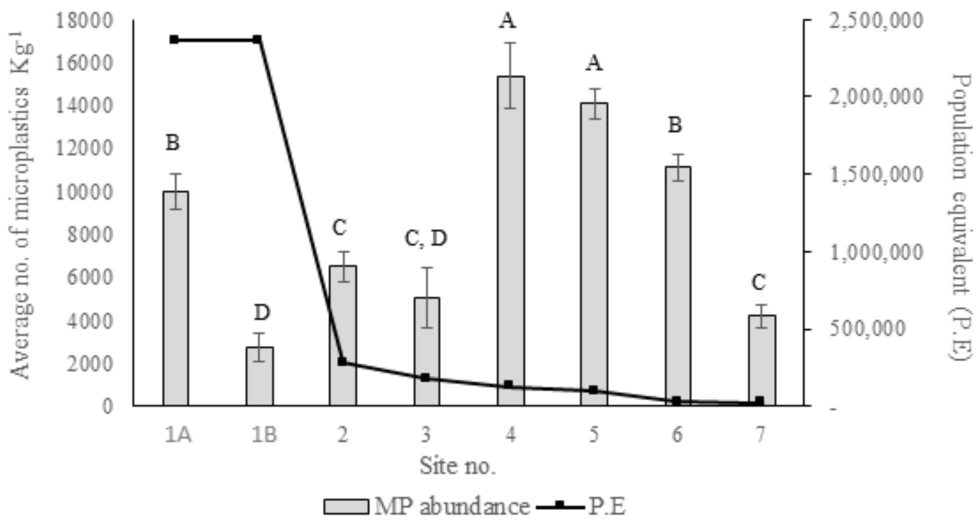

Figure 1. Average abundances and corresponding population equivalents of microplastics at 7 sites. Sites sharing the same letter are not significantly different (Mann- Whitney-U test, p > 0.005)

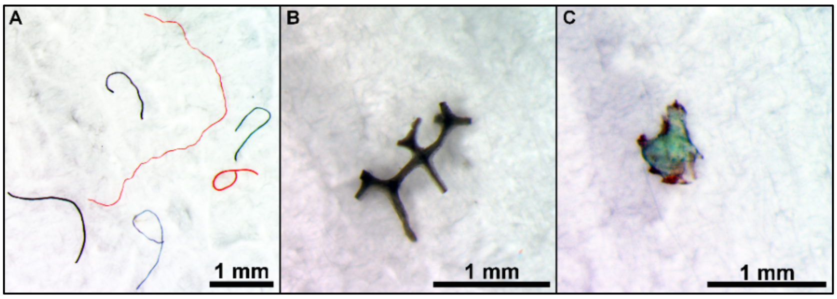

Figure 2. Stereomicrograph of mircoplastics fibres (A), other (B) and fragment (C).

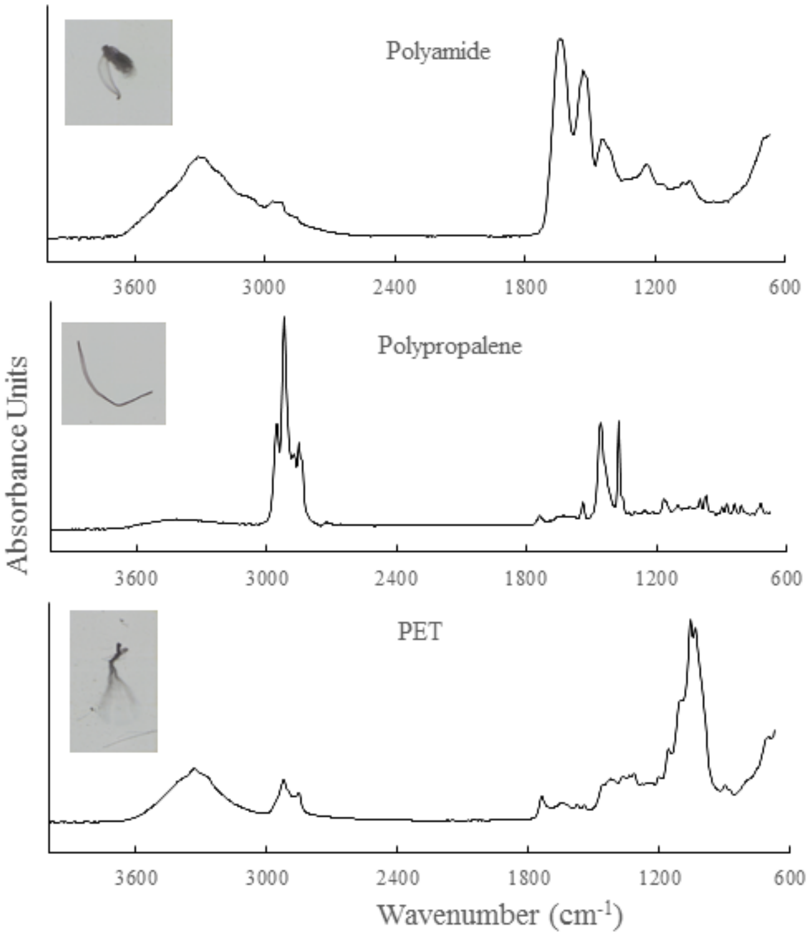

Figure 3. Fourier Transform Infrared Spectroscopy (FTIR) spectra within specimen photographs of polyamide, polypropalene and Polyethylene terephthalate (PET).

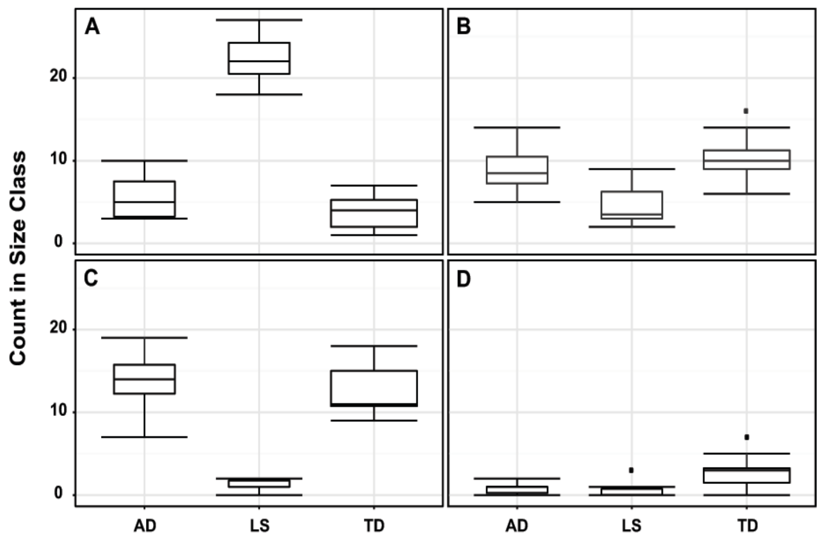

Figure 4. Abundance of microplastics in different size classes (**A**: 250-400 μm, **B**: 400-600 μm, **C**: 600-1000 μm, **D**: 1000-4000 μm) as a function of treatment type.

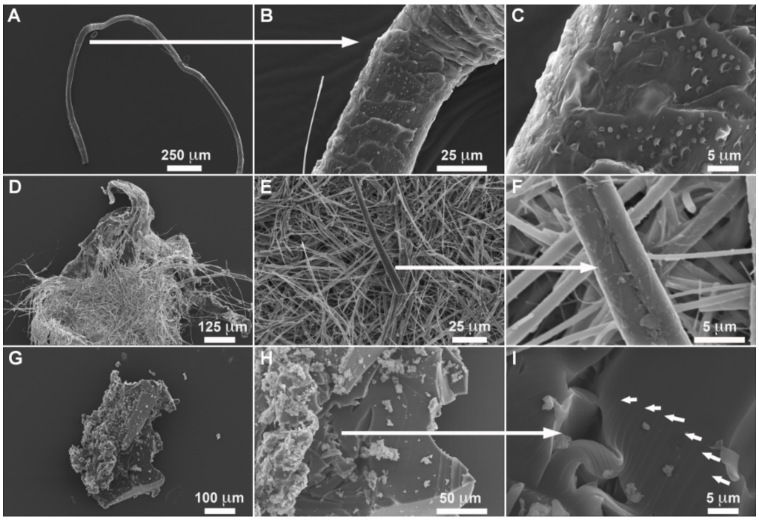

Figure 5. Diversity in morphology and surface texture of microplastics isolated from treated sewage sludge. Scanning electron micrographs of fibrous particle from thermally dried (TD) biosolids (A-C). Multi fibrous particle from lime stabilised (LS) biosolids (D-F). Overview of non-fibrous particle from anaerobically digested (AD) biosolids (G-H). Presence of lamellae or cleavage planes (arrow heads) on microplastic surface (I).

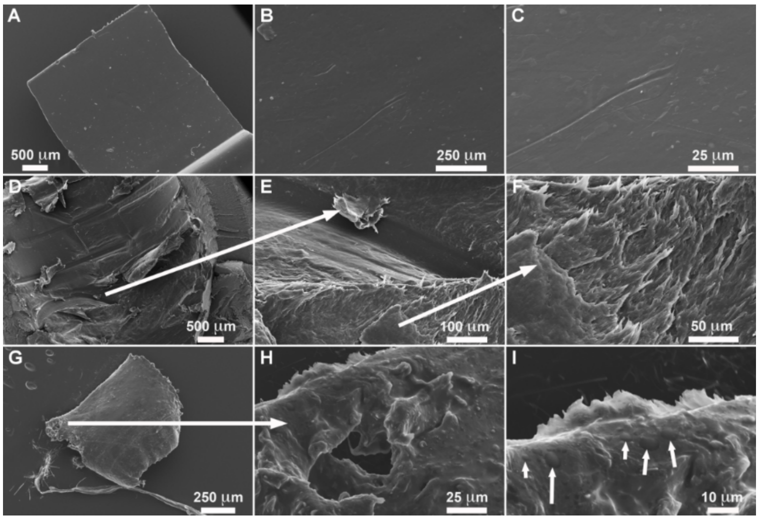

Figure 6. Morphological and surface texture comparison between pre-treatment high density polyethylene (HDPE) and HDPE particles isolated from treated sewage sludge. Scanning electron images of pre-treatment HDPE (A-C) showing smooth non-degraded surface. Scanning electron micrographs of HDPE particle from lime stabilised (LS) biosolids (D-F) showing altered and weathered surface texture. Scanning electron micrograph of HDPE particle from thermally dried (TD) biosolids (G-I) with evidence of blistering effect (arrow heads) on polymer surface (I).

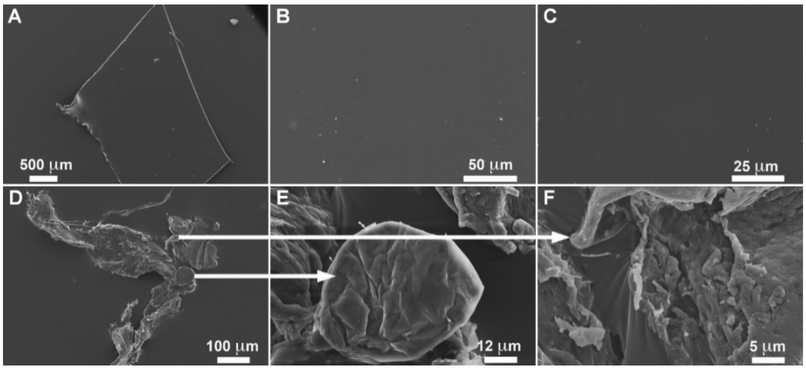

Figure **7**. Morphological and surface texture comparison between pre-treatment polyethylene (PE) and PE particle isolated from sewage sludge. Scanning electron images of pre-treatment PE (A-C) with unaltered surface. Scanning electron micrographs of PE particle from thermally dried (TD) biosolids (D-F) showing wrinkling and fracturing of polymer surface.